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SOLUBLE CD40 LIGAND IN DEMENTIA

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ABSTRACT

Some 15-20% of the population over the age of 65 years suffer from dementia, currently one of the leading causes of death behind cardiovascular diseases, cancer and cerebrovascular diseases. The major forms of dementia share in common overactivation of the CD40–CD40-L complex, leading to high levels of proinflammatory cytokine production by immune cells of the central nervous system (CNS), including microglia and astrocytes. Consequently, both neuronal survival and signaling are negatively affected, leading to the characteristic progressive loss of higher cortical functions. We have reviewed the literature concerning the involvement of this complex in the pathology of three major forms of dementia: Alzheimer's-type, HIV-associated and vascular dementia. This is followed by a discussion of current preclinical and clinical therapies that may influence this interaction, and thus point the way toward a future neuroimmunological approach to inhibiting the effects of CD40–CD40-L in neuropsychiatric disease.

INTRODUCTION

As the number of elderly individuals continues to rapidly increase, dementia has led to a massive socioeconomic burden which is projected to worsen. Specifically, some 15-20% of the population over the age of 65 years suffer from dementia (1, 2). Its presentation is heterogeneous, as it is caused by multiple disorders. Alzheimer's disease (AD) and vascular dementia (VAD) are the two main causes, affecting 25-45% and 15-35%, respectively, of all patients suffering from dementia (3). Among dementias where brain infectious viruses are etiological, HIV-1-associated dementia (HAD) is the most common cause of dementia (4).

All three of these disorders share a common neuropathological hallmark: microglial activation associated with increased CD40 pathway signaling. The interaction of membrane-bound CD40 (expressed by innate immune cells, including microglia) with its cognate CD40 ligand (CD40-L) plays a critical role in microglial phenotypic transformation from a ramified (resting) to an activated macrophage morphology (5), and enhances the surface expression of inflammatory markers (6, 7). These cell-surface molecules in turn enhance the inflammatory cycle. Conversely, low levels of molecules involved in antigen presentation, including CD40, are expressed by resting microglia (6, 8). CD40 is a 45- to 50-kDa type I integral membrane glycoprotein and a member of the tumor necrosis factor receptor (TNFR) superfamily. Landmark studies originally identified CD40 on B lymphocytes, where it was shown to mediate T-cell-dependent B-cell activation and differentiation. It is found on most immune and some nonimmune cells, including macrophages, dendritic cells, endothelial cells, smooth muscle cells and B cells, as well as astrocytes and microglia (9-15). CD40-L, also known as CD154, is a trimeric 33-kDa type II membrane glycoprotein that is predominantly expressed by activated T and B cells, astrocytes and platelets (13, 16, 17). Importantly, CD40-L also occurs in a soluble, secreted form (sCD40-L) that retains biological activity to bind and activate membrane-bound CD40 (18, 19).

It is now generally accepted that the CD40–CD40-L interaction is central to the pathophysiology of risk factor-related vascular damage (20, 21). Cerebral perfusion abnormalities are seen in the earliest phases of AD (22) and are a well-established hallmark of VAD. This is not surprising, as the majority (> 95%) of circulating CD40-L is bound to platelets. After activation, CD40-L is rapidly translocated to the platelet surface and is then cleaved, giving rise to sCD40-L over a period of minutes to hours (20). Additionally, circulating sCD40-L is an independent predictor of restenosis following percutaneous transluminal angioplasty (23). The clinical association between sCD40-L and VAD is also strengthened by evidence of sCD40-L engagement of CD40 on endothelial cells, resulting in various proinflammatory responses, including the expression of adhesion molecules and tissue factors, the release of cytokines/chemokines and the expression of vessel-remodeling metalloproteinases (24, 25). The interaction between sCD40-L or CD40-L and CD40 activates various signaling cascades via the TNFR-associated factor (TRAF) family members (26). As sCD40-L also negatively

affects vascular endothelial cell functions (24, 27), it has even further potential to interfere with microcirculation, increasing the risk of VAD development.

HIV-ASSOCIATED DEMENTIA

HIV-1 enters the central nervous system (CNS) early after infection, persists in the brain compartment for decades and generates clinical symptoms that include behavioral, motor and cognitive deficits in a significant proportion of infected patients (28). Together these clinical features are referred to as HIV-associated dementia, or HAD. The severity of HAD is strongly correlated with the number of activated macrophages and microglia within the basal ganglia and frontal lobes (29). Indeed, the hallmarks of HAD are HIV-1-infected macrophages and microglia in the brain, the formation of syncytial, multinucleated giant cells (30-32), perivascular infiltrates of inflammatory cells, astrogliosis and neuronal damage and/or loss – especially in the hippocampus, basal ganglia and cerebral cortex (4).

The introduction of highly active antiretroviral therapy (HAART) in the mid-90s dramatically reduced mortality associated with HIV-1 infection (32); however, it fails to eradicate the virus (33). Furthermore, many HAART drugs fail to adequately penetrate the CNS – likely allowing virus to replicate in the brain (4). The use of HAART has resulted in a significant decline in the incidence of HAD, but the prevalence has actually risen as HIV-infected individuals experience longer lifespans (34). Currently, more subtle forms of CNS disease, known as minor cognitive motor disorder (MCMD), have become more common in HIV-1 patients receiving HAART (35).

Productive CNS infection by HIV occurs primarily in perivascular macrophages and microglia, but not neurons. Accordingly, neuronal damage and death occur via indirect pathways relying on the intrinsic toxicity of secreted viral proteins and the excessive release of inflammatory mediators by infected/activated cells (36). Two such inflammatory mediators that work in synergy are sCD40-L and the HIV-1 transactivating protein Tat (37, 38). Both play important roles in HAD and HIV-related CNS disorders because of their ability to chemoattract specific subsets of leukocytes that may be infected, and because they activate resident CNS cells (37, 39-41), which in turn fuel excessive release of inflammatory mediators. Along these lines, Sui et al. demonstrated that CD40-L synergizes with Tat protein, causing elevated TNF-α production by human monocytes and microglia. This occurred via Tat upregulation of CD40 expression on these cells, sensitizing them to CD40-L. Studies by this same group on the neurotoxicity of Tat/CD40-L-exposed monocyte conditioned medium (CM) revealed a significantly higher level of neurotoxicity than CM from monocytes exposed to Tat or CD40-L alone. Furthermore, immunodepletion of TNF-α from CM nearly completely mitigated neurotoxicity (38).

Untreated HIV-1 infection is also associated with an elevated release of soluble markers for both endothelial and platelet activation, including sCD40-L (38, 40, 42-44). Others include the possible HIV-1 neurotoxin platelet-activating factor (PAF) (45), as well as soluble VCAM and von Willebrand factor (42, 43). HAART has been shown to reduce the latter two markers in HIV-1-infected patients (46, 47), but to have no effect on soluble platelet activation markers, including PAF acetylhydrolase (48) and sCD40-L (44).

Similar to HIV-1 Tat protein, sCD40-L also interacts with the chemokine IL-8 (also known as CXCL8). IL-8 is a chemotactic activating factor for primarily neutrophils (49, 50), and is also a chemoattractant and activating factor for T cells (51) and monocytes (52). IL-8 expression is increased in HAD brain tissue and plasma compared with uninfected tissue (53, 54), colocalizes with CD68/CD40-positive cells in HAD brain tissue, and stimulates HIV-1 replication in macrophages and T cells (55, 56). Soluble CD40-L is integral to the actions of IL-8, as microglia only secrete it after ligation of surface CD40 with CD40-L (55). Furthermore, sCD40-L is present at elevated levels in the plasma and cerebrospinal fluid (CSF) of HIV-1-infected individuals with cognitive impairment compared to HIV-1-positive subjects with normal cognitive function (38). Moreover, in at least one study, HAART administration has been positively associated with an increase in serum sCD40-L levels (40). Perhaps these phenomena may contribute to the increased prevalence of HIV-1-related cognitive impairment in the era of HAART.

ALZHEIMER'S DISEASE

Deposition of β-amyloid peptide (Aβ) into senile plaques and intracellular accumulation of neurofibrillary tangles (principally composed of phosphorylated tau protein) are the hallmark features of Alzheimer's disease (AD), the most common form of dementia (57, 58). Also, cerebrovascular dysregulation likely contributes to the development of the neurodegeneration that characterizes this dementing illness (59). As in HAD, a continuous inflammatory cycle exists in the AD brain, marked by chronic, low-level secretion of proinflammatory cytokines and acute-phase reactants around amyloid deposits (60). The inflammatory response is largely mounted by reactive glia, principally microglia, which are in an activated state surrounding "senile" plaques that are largely comprised of deposited 40- to 42-amino-acid Aβ peptides. We and others have demonstrated that microglia are activated at a low level by Aβ exposure in vitro, and that costimulation with interferon gamma (IFN-γ) or CD40-L synergistically affects microglial activation (61, 62). CD40 protein induction on microglia and vascular endothelial cells occurs in the presence of low levels of more soluble forms of Aβ, suggesting that this may occur prior to Aβ deposition (62, 63).

The pattern of expression of both CD40 and CD40-L is altered in the brains of AD patients, as well as in several animal models of AD (17, 64). It has recently been suggested that sCD40-L could be a potential AD biomarker, thereby allowing early identification of subjects requiring treatment. Specifically, it was found that patients with plasma sCD40-L concentrations of $\geq$ 6.0 ng/mL experience a 3-fold greater risk of suffering cognitive decline in the following 2 years (65). Importantly the subjects in this study were free of any drugs that could potentially affect sCD40-L, including statins, antioxidants and/or anti-inflammatory agents. Because of the close risk association between sCD40-L and both AD and vascular disease (66), the study could not completely exclude the possibility that silent brain infarcts could have occurred in some patients and contributed to a worse AD clinical presentation (67).

Aβ-induced CD40 is inserted into the cell membrane and is constitutively expressed at low levels on vascular smooth muscle and endothelial cells (63, 66, 68). Thus, not surprisingly, the risk relationship between sCD40-L and AD mirrors the positive correlation

between sCD40-L levels and vascular disease, as well as sCD40-L levels and the risk of cognitive impairment in HIV infection. Furthermore, endothelial cells are a key component of the blood–brain barrier, suggesting that there could be alterations of this physiological unit under conditions of increased sCD40-L expression or function. Accordingly, it was previously demonstrated that Aβ increases both CD40 mRNA transcription and CD40 protein expression in cultured human vascular endothelial cells within 48 h of exposure (63, 68). Interestingly, these findings further suggest that factors which increase the risk for vascular disease also increase the risk for AD (69, 70). For example, brain ischemia induced by infarction is associated with worse cognitive decline in AD (71), and alterations in cerebral blood flow precede the onset of the disease (72). Such findings suggest that the role of platelet-derived sCD40-L should be considered in the context of AD neuropathogenesis. This concept is supported by findings from Sevush et al., which showed multiple abnormalities in platelet activation in AD patients (73). Taken together, the soluble or membrane-bound CD40-L and CD40 on smooth muscle and endothelial cells seem to work with Aβ peptides to promote a feed-forward, self-perpetuating inflammatory cascade in both the vascular wall and brain parenchyma, which in turn may initiate AD pathogenesis and/or propagate its progression (63, 68, 74, 75).

A second route by which sCD40-L may modulate AD pathogenesis is via T cells, which, like microglia, are increased in number and demonstrate altered activated profiles both in the circulation and brain parenchyma of AD patients (76). Activated T cells are one of the key sources of membrane-bound CD40-L and may also shed sCD40-L, and it has been shown that intercellular adhesion molecule 1 (ICAM-1) facilitates the migration of T lymphocytes across the brain endothelium (77). Ligation of endothelial cell CD40 by sCD40-L may upregulate ICAM-1 (78), pointing to a mechanism by which sCD40-L derived from brain-penetrating T lymphocytes might also promote neuroinflammation (76).

Finally, we and others have shown that the CD40–CD40-L complex also acts synergistically with Aβ peptide to promote proinflammatory activation of microglia, leading to brain inflammation and neuronal injury. Specifically, when cultured microglia are coincubated with Aβ peptide and CD40-L in the presence of primary neurons, the microglia synergistically secrete TNF-α, resulting in neuronal death (62). Furthermore, the expression of genes involved in amyloid precursor protein (APP) processing and tau phosphorylation is disturbed in cultured human microglia following challenge with CD40-L (62, 79-81). Laporte et al. demonstrated that mice deficient in CD40 and carrying the human "Swedish" mutant APP (APP_{sw}; a mutant form of APP that increases the production of Aβ) show significantly less microgliosis, astrocytosis and Aβ load compared to CD40-sufficient littermates (82). This work validates earlier work in our laboratory that demonstrated that anti-CD40-L treatment causes a reduction in cerebral Aβ pathology and improved performance in several cognitive tasks in the double transgenic PSAPP mouse model expressing mutant human presenilin-1 and human APP_{sw} (unpublished results). Further supporting this line of evidence, a CD40-L-neutralizing antibody reduced AD-like pathology and improved cognition in the double transgenic PSAPP AD mouse model (82, 83). Specifically, Todd Roach et al. showed that PSAPP mice given a regimen of anti-CD40-L antibody commencing at an age when initial Aβ deposition occurs had superior spatial and non-spatial memory compared to control PSAPP mice (83).

FUTURE APPLICATIONS FOR sCD40-L AS A BIOMARKER AND TREATMENT TARGET FOR DEMENTIA

Biomarker and therapeutic target

Many groups, with the goal of identifying objective quantitative markers of neuropsychiatric illnesses, have measured CSF cytokines in various psychiatric conditions. These types of studies have been plagued by problems of reproducibility, as the literature reporting intrathecal or circulating cytokine levels in such diseases is often discordant. This could be due to the relatively small sample sizes used, the nature of cytokine activity (characterized by a short half-life) and signaling that occurs mainly in an autocrine or paracrine fashion. Additionally, cytokines can be buffered by soluble receptors in the periphery, which bind to the cytokine and can therefore obscure detection. Although the initial event(s) triggering the neurodegenerative processes in VAD, AD, HAD and other dementing illness are likely different, it is reasonable to hypothesize that such event(s) may converge on a common cascade of proinflammatory cytokine production in response to neurodegenerative changes, whether it be from a ruptured vascular plaque, HIV-1 proteins or neuritic Aβ plaques. The cytokines released in the CNS may, in turn, act to amplify certain neuropathological events. However, it is worth noting that a common cytokine release pattern "footprint" in the CSF has yet to be identified for different neurodegenerative diseases (84).

Another approach that we present here is to measure peripheral levels of proinflammatory molecules such as sCD40-L as an index of CNS changes associated with neurodegenerative diseases. A recent study by Ray et al. took an unbiased proteomics approach to screen 120 proteins and identified a panel of 18 markers (many of which mediate immune/inflammatory events) that correctly identify AD approximately 90% of the time (85). Along these lines, the finding of increased peripheral sCD40-L levels in HAD and AD could potentially be of clinical relevance as a biomarker, as the assessment of sCD40-L plasma levels might allow for early identification of those at risk for cognitive impairment, such that therapy could be initiated prior to the onset of full-blown disease.

Regarding the focus on the CD40 pathway as a therapeutic target for dementia, epidemiological studies suggest that statin administration might protect against the development of AD (86). Interestingly, we showed that the commonly used cholesterol-lowering agent lovastatin suppresses IFN-γ-induced CD40 expression. Additionally, it markedly inhibits IFN-γ-induced phosphorylation of JAK/STAT1 (Janus kinase/signal transducer and activator of transcription 1), a key intracellular proinflammatory signaling pathway. Furthermore, lovastatin is able to suppress microglial TNF-α, IL-1β and IL-6 production promoted either by IFN-γ or by Aβ peptide challenge in the presence of CD40 stimulation, and lovastatin markedly blunts CD40-mediated inhibition of microglial Aβ clearance from the brain (87).

Other statins have been shown to have somewhat similar effects. In a small pilot study, patients with mild to moderate AD who were

administered 80 mg of atorvastatin daily over 1 year experienced significant clinical improvement (88). As sCD40-L is involved in both the activation of microglia and the induction of vascular inflammation, this clinical benefit may be secondary not only to the effect of lowering cholesterol on the reduction of vascular risk factors, but also to reduced Aβ deposition in brain parenchyma, as demonstrated in our experimental models (87). Individuals enrolled in a randomized, controlled trial testing two anti-inflammatory agents for the primary prevention of AD (Alzheimer's Disease Anti-inflammatory Prevention Trial; ADAPT) were allowed the elective use of statins to assess whether their use was associated with a reduced incidence of AD. It was found that elective statin use was associated with a significantly reduced risk of AD after adjustment for age, gender, education and apolipoprotein E (Apo-E) genotype. The findings were similar when comparing the use of lipid-lowering agents (statins and nonstatins) to the use of non–lipid-lowering agents (89). An international, multicenter, double-blind, randomized trial (LEADe, Lipitor's Effect in Alzheimer's Dementia) is under way in 641 subjects with mild to moderate AD treated with atorvastatin in addition to donepezil to definitively evaluate the potential of statins in AD (90).

Additionally, because statins have lipid-independent anti-inflammatory properties, including reduction of circulating sCD40-L (91, 92), these compounds may provide multiple mechanisms for therapeutic benefit in neurodegenerative diseases. Most recently, the Rotterdam study addressed the conflict between some cross-sectional reports suggesting that statin users are less likely to develop AD with prospective studies providing inconsistent evidence. In this prospective, population-based study (N = 6,992) patients were followed for 12-15 years for the incidence of AD. A distinction was made between statins, lipophilic and hydrophilic statins, and nonstatin cholesterol-lowering drugs. Compared with a total lack of use of cholesterol-lowering drugs, statin use was associated with a decreased risk of AD (hazard ratio [HR]: 0.57; 95% confidence interval [CI]: 0.37-0.90), but nonstatin cholesterol-lowering drug use was not (HR: 1.05; 95% CI: 0.45-2.44). The authors concluded that in the general population, statin use, but not the use of nonstatin cholesterol-lowering drugs, was associated with a lower risk of AD compared with no use of cholesterol-lowering drugs, independent of the lipophilicity of the statins (93). Taken together, these results suggest that statins, through both CD40-dependent and -independent pathways, may be a viable therapeutic avenue for neurodegenerative diseases, including AD.

Human umbilical cord blood cell transplantation

An additional therapeutic avenue, which relies in part on suppression of CD40 signaling, is human umbilical cord blood cell (HUCBC) transplantation. HUCBCs have unique immunomodulatory potential. Previous experiments in our laboratory have shown that these cells mitigate AD-like pathology after infusion into the PSAPP mouse model. Specifically, we observed a marked reduction in parenchymal plaques and associated astrocytosis following multiple low-dose infusions of HUCBC. HUCBC infusions also reduced cerebrovascular Aβ deposits in the Tg2576 AD mouse model. These effects were associated with suppression of the CD40–CD40-L interaction, as evidenced by decreased circulating and brain sCD40-L, elevated systemic immunoglobulin M (IgM) levels, attenuated CD40-L-induced inflammatory responses and reduced surface expression of CD40 on microglia. Interestingly, CD40 deficiency abolishes the effect of HUCBC on elevated plasma Aβ levels, demonstrating the requirement for intact CD40–CD40-L signaling for this beneficial effect. Moreover, microglia isolated from HUCBC-infused PSAPP mice demonstrated increased phagocytic clearance of Aβ, and sera from HUCBC-infused PSAPP significantly increased microglial phagocytosis of the Aβ peptide and inhibition of IFN-γ-induced microglial CD40 expression. Increased microglial phagocytic activity in this scenario was inhibited by the addition of recombinant CD40-L protein, again delineating the key role of the CD40 pathway in this effect (94).

Aβ vaccination

Aβ immunization efficiently reduces amyloid plaque load and memory impairment in transgenic mouse models of AD (95-97). Active Aβ immunization, accomplished by administration of Aβ peptide plus adjuvant, has also yielded favorable results in a subset of AD patients (98). However, a small percentage (~6%) of patients receiving the AN-1792 active Aβ vaccine developed severe aseptic meningoencephalitis associated with brain inflammation and infiltration of T cells. We recently demonstrated that genetic or pharmacological interruption of the CD40–CD40-L complex enhances the efficacy of $A\beta_{1\text{-}42}$ immunization in reducing cerebral amyloidosis in the PSAPP and Tg2576 mouse models of AD. Potentially deleterious proinflammatory immune responses seen in the previous Elan/Wyeth AN-1792 trial (cerebral amyloid angiopathy and cerebral microhemorrhage) were reduced or absent in these combined approaches when tested in animals, raising the possibility that this combined approach may be both safe and effective in AD patients. In particular, pharmacological blockade of CD40-L decreased T-cell neurotoxicity to Aβ-producing neurons. Furthermore, reduction of cerebral amyloidosis in Aβ-immunized PSAPP mice homozygously deficient in CD40 was correlated with anti-inflammatory cytokine profiles and reduced plasma sCD40-L. These results suggest that CD40–CD40-L blockade promotes anti-inflammatory cellular immune responses, likely resulting in the promotion of microglial phagocytic activity and Aβ clearance, without the generation of neurotoxic Aβ-reactive T cells. Thus, combined approaches of Aβ immunotherapy and CD40 pathway blockade may provide for a safer and more effective Aβ vaccine in the future (99).

CONCLUSIONS

As the number of elderly individuals continues to increase, the prevention and treatment of dementia must be improved, as the illness is currently one of the leading causes of death after cardiovascular diseases, cancer and cerebrovascular diseases (2). Although the presentations of AD, VAD and HAD may be heterogeneous, we have attempted to highlight the importance of targeting inhibition of the CD40–CD40-L complex since all three of these disorders share a common neuropathological hallmark: microglial activation associated with increased CD40 pathway signaling.

DISCLOSURE

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